ON THE

ALTERATIONS IN THE RED GLOBULES IN MALARIA INFECTION,

AND

ON THE ORIGIN OF MELANÆMIA.

MEMOIR BY

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Of all the changes which malarious infection determines in the human organism, the most characteristic is that which takes place in the blood, and is called *melanæmia.** This dyscrasia consists in the presence in the blood of brownish yellow or black pigment, rarely free, generally collected in the white cell elements. The existence of pigment in the blood is easily verified by examining a very thin film ; and in the bodies of persons who have died from pernicious fevers a great abundance of pigment is found, free, or inclosed in the cell elements, in the blood vessels of the spleen, marrow, brain, kidneys, &c. When the melanæmia has ceased, there remains *melanosis* of the organs, *i.e.*, deposit of granules and pigment masses around the vessels and perilobular connective tissue of the liver, and in like manner in and around the lymphatics. The *melanosis* witnesses to the antecedent *melanæmia.*

The origin of this pigment has been by almost all authors derived from the colouring matter of the blood, but there has been no agreement as to the site or manner of its formation, as will be seen from the following *résumé* of the history of melanæmia.

The ancient school of physicians† held that sometimes black material was formed in the spleen and blood of the portal vein, capable of giving rise to diseases. The "black bile" formed an

* Melanæmia, so far as we now know, occurs only in malarious infection, and possesses characters so special that one cannot conceive how they have been confounded by some authors with the hæmorrhagic diathesis, Addison's disease, &c.

† Frerichs, *Clinic of Liver Diseases.*

essential part of their humoral pathology, and Galen held that it accumulated in the spleen and caused engorgement of the blood vessels, destruction of the intestines, and serious disturbances of the nervous system. Leaving on one side, however, the views of the ancients about "black bile," we find that from the last century it began to be noticed that some organs showed a black or blackish colour in grave malarious fevers, so that Lancisi,* for example, speaking of the changes met with in persons dead from malaria, says: *Primum in iis qui ob tertianos perniciosas occiderunt, ingens malorum sedes sub aspectum venit in abdomine ubi omnia livida, et potissimum hepar subfusci, ac bilis cystica tri coloris passim occurrerunt.*

Mekel, however, was the first to observe pigment in the blood, and held that it passed from the spleen into the blood.

Virchow† discovered pigmented elements in the blood and spleen of a man who died of dropsy following repeated attacks of malarious fever. He believed the pigment to be derived from the spleen, and persisted in affirming melanæmia to be a dyscrasia of that organ.

Tigri saw and described melanosis of the spleen as "black spleen."

Frerichs‡ accurately described melanæmia and its effects. He observed black granules, pigment cells, masses of black granules held together by hyaline substance, delicate or dense, but always without fixed shape, and pigment masses, sometimes cylindrical with parallel facets, surrounded by a ring of pellucid substance. He believed the pigment formed in the spleen, and passed into the general circulation. In proof of this he adduced the following arguments:—

a. Pigment is found in the healthy spleen. *b.* In melanæmia the spleen is always more largely charged with pigment. *c.* Pigmented epithelium of the spleen is found in the general circulation.

He believed that the liver shared in this power of forming pigment. He confirmed the view of Virchow that in intermittents pigment forms in the spleen and passes into the circulation.

Colin§ holds, on the other hand, that this formation is not

* *De noxiis paludum effluviis eorumque remediis.* Roma, 1727.

† *Cellular Pathology.* Berlin, 1881.

‡ *Loc. cit.*

§ *Traité des fièvres intermittentes.* Paris, 1870.

confined to the spleen but occurs also in other organs, and has no specific character, because it happens, for example, in the mesenteric lymphatics in typhus and dysentery. In malarious fever, however, the destruction of the red globules is more rapid and remarkable than in other infectious diseases.

Mosler* is inclined to admit the doctrine of Virchow and Frerichs, of the primitive formation of pigment in the spleen. He holds that the peculiar structure of this organ predisposes to this pigment formation, that is to say, that the blood flowing from the capillaries into the intermediate vessels often stagnates, so that conglomerations of red globules occur, which are gradually converted into pigment.

Arnstein † rejects the doctrine of Virchow, &c., and maintains that the pigment is formed in the circulating blood during the fever *accès*. He says the pigment is found free in the blood when the fever has proceeded for a short time, and that in the majority of cases it is met with in the white cells. He examined the organs chiefly melanotic. In the spleen he found pigment round the arteries, capillaries, and veins, and in all these enclosed within the white cells. In recent cases only he found it in other organs.

He maintains that the view of Virchow and Frerichs is destitute of any support from facts. On the other hand, he regards the melanæmia as primary, and the melanosis secondary; and this chiefly because the melanæmia can only be found shortly after the febrile attack (which is unintelligible if the melanosis of the spleen was primary), and because the disposition of pigment in the circulating blood corresponds perfectly to what happens when colouring matter like cinnabar is introduced into the circulation. According to Arnstein, therefore, the red globules are destroyed during the fever paroxysm, the pigment formed from them is immediately taken up by the white globules, which stagnate in the veins and capillaries of those organs in which the current is feeble, viz., the spleen, bone marrow, and liver. The pigmented white cells are deposited in the tissues of those organs, whilst they linger longer in the capillaries of the liver. As to the mode of formation of the pigment he says he knows nothing, because the process of destruction in the red globules is too rapid to be

* Ziemssen, *Handbuch*, T. 8, Part. Milzkrankheiten, 1874.

† *Bemerkungen über Melänemie und Melanose.* Virchow's Archives, T. 61.

followed. He admits that it may occur in the serum of the blood from the hæmoglobin issuing from the red globules. He does not believe it is formed in the white cells, according to what Longhaus has observed in hæmorrhage, because free pigment is found in the circulating blood and globuliferous cells are not. The latter, however, are found in the spleen and marrow, but are few in comparison with the enormous quantity of pigment which exists in the blood.

Mosler,* in a later work, does not agree with Arnstein, that the pigment is only found after a febrile *accès*, and that it is erroneous to hold that its formation takes place in the spleen. He cites the case of a man affected with malarious fever, in whom the pigment was present, not only during, and shortly after the febrile paroxysm, but several months afterwards.† He persists in maintaining that it takes place only in the spleen, or that at least it happens in this organ more rapidly and abundantly in consequence of severe congestions.

Lanzi and Terrigi‡ notice the great analogy between the pigment granules found in the blood and organs in melanæmia, with those which exist in marsh soils, and especially in the cells of marsh algæ, and suspect that they are identical.

Kelsch,§ in a very accurate work, sums up the observations made in 1881 on the sick in the Hospital at Philippeville. He was chiefly occupied with the histology of the blood and organs. He found in the blood a diminution in the number of red globules computed by the method of Melassez. He observes that few maladies produce so rapid and serious an oligocitæmia as malaria; twenty or thirty days of quotidian or tertian suffice to lower the number of red globules in a C. mm. from five millions to one million, or even less than half a million. The rate of diminution in the number of red globules has its maximum at the outbreak of the disease, and goes on diminishing as the oligocitæmia increases, and the febrile attacks become more distant. In the cachexia the number of red globules becomes almost stationary. Kelsch found, in addition to this diminution of red globules, the presence in the blood of pigment granules, free or inclosed in

* *Über das Varkommen der Melanëmie.* Virchow's Archives, T. 61.

† Cases, analogous to this narrated by Mosler, are seen in our hospitals. See *Raggualgio di due Turni di Clinica Medica di Roma di Professor C. Maggiorani*, 1873.

‡ *La Malaria ed il Clima di Roma.* Rome, 1877.

§ *Contribution à l'anatomie pathologique des maladies palustres endémiques.*

hyaline masses, but oftener in the white cells. The melaniferous elements enclose 3—6 granules, sometimes massed in a series like a crown in the periphery of the cell. Besides these, others are met with which present a brownish reflection accentuated in the marginal zone, in which are sometimes found very fine black granules, and in the blood of the splenic and portal veins he observed the presence of melaniferous cells, very variable in form and size, viz., spherical, polyhedric, ovoid, elongated, biscuit-shaped, &c. From observations of the organs he comes to the conclusion that melanæmic pigment is deposited absolutely in the same way as an artificial pigment, injected into the veins, either free or enclosed in the cells. As to the mode of formation of pigment he rejects the doctrine of Virchow and Frerichs.

In one case of "perniciosa fulminante" he found little pigment in the spleen, but the blood very rich in it. He therefore believes it is formed in the circulating blood. He is, however, unable to accept that some melaniferous cell elements represent the stroma of decolourized blood globules with pigment granules, formed at the expense of the hæmoglobin, because he has not met with the intermediate stages of this retrograde metamorphosis. Nor does he admit the intracellular formation of the pigment, with Longhaus, because it is also met with free in the blood. He therefore takes refuge in the hypothesis, that the melanic material exists in the serum in a state of solution, and proceeds from the destruction of the red globules; this material precipitates, when the blood is saturated with it, under the form of granules, which are quickly included in the white cells, as happens when cinnabar is injected into the blood.

One of us (Marchiafava*) studying the splenic pulp and bone marrow of melanæmic children, found red globules generally inclosed in white cells (globiferous cells), which exhibited a varied colouring, which passed from a brownish yellow to a more or less intense black, presenting the appearance of black spherules of a diameter somewhat less than a red globule. He concludes, therefore, that the red globules do not give rise to the formation of pigment after their destruction, but that the conversion of hæmoglobin into pigment occurs gradually within them.

Klebs and Tommasi-Crudeli,† studying the changes in the

* *Commentario Clinico di Pisa.* Gennaio, 1879.
† *Sulla natura della melaria.* 1879.

spleens of rabbits in which they had produced malarious fevers artificially, found red globules, at first nickel-coloured and preserving their discoid shape, afterwards of a black colour, and inclosed singly or in groups in the white cells, with the destruction of which they became free and reduced into masses. They conclude that the conversion of hæmoglobin into black pigment takes place in the red globules while they yet retain their semi-fluid consistence.

Tommasi-Crudeli* also holds that this degeneration of red globules occurs throughout the whole vascular system, and chiefly in the vessels of the spleen and bone marrow. He says that in melanæmia takes place a *necrobiosis* of red globules, produced by a specific degenerative atrophy of their protoplasm.

Afanassiew† made his observations on the sick amongst the troops in the last Russo-Turkish war. He describes the affections of the spleen, kidneys, liver, and brain in those dead from malaria. His description is in accord with other observers as regards the spleen, liver, and kidneys. In the brain he found distinct cloudiness of the protoplasm of the nerve cells, with enlargement of the pericellular spaces; the capillary walls sometimes granular; the endothelium swollen; pigment granules in the lumen of the vessels, in the endothelial cells, in the perivascular areas. He describes such granules when they are isolated, and not formed into masses, as perfectly round, of equal size, and having a shining brown centre. He doubts their origin from the colouring matter of the blood; suggests that they may have another origin; finds that they have an analogy with the *cromigenous micrococci* of Cohn, and suspects their parasitic nature. With the history of melanæmia are connected some recent researches of Laveran and Richard.

Laveran‡ holds that the pigmented elements described in part by Kelsch, and different from melaniferous white cells, represent parasitic elements. He describes three forms of these pigmented elements, which he holds to be of parasitic nature. 1st. Elongated elements, threadlike at the ends, almost always curved like a crescent length 0·008—0·009 mm.; mean breadth

* *Institutes of Pathological Anatomy.* Turin, 1882—4.

† *Beitrag zur pathologie der malaria infection.* Virchow's Archives, 1881.

‡ *Nature parasitaire des accidents de l'impaludisme.* Paris, 1881. Comptes rendus (1882).

0·003; their contour marked by a very fine line; the body transparent except in the centre, where there exists a spot formed of blackish granules; often on the concave side is seen a fine line uniting the two extremities of the half moon.

2nd. Spherical transparent bodies, of the mean diameter of a red globule containing pigment granules, which in repose often describe a very regular circle, and when in movement are actively agitated. Sometimes at the periphery of these elements are observed very fine filaments, which are apparently inserted into them, are animated by very rapid movements, and have the free extremity slightly swollen. Such filaments may be detached, and move freely in the midst of the red globules.

3rdly. Spherical elements, of irregular form, transparent and finely granular, diameter 0·008—0·010 mm., containing pigment granules disposed very irregularly at the periphery, sometimes collected in the centre, sometimes at a point of the periphery itself.

There are also found in the blood, according to Laveran, spherical transparent elements, containing movable and immovable pigment granules, elements of smaller diameter than the preceding, isolated or united, and adhering to the red discs and the leucocytes.

Laveran believes that these pigmented bodies represent different stages of an elementary parasite, which he is unable to pronounce to be animal or vegetable, which exists in an encysted condition, and in its perfect form becomes free as a *movable filament.*

Besides these, Laveran has observed in the blood shining, round, movable bodies, without specific character; melaniferous leucocytes, red globules which appear perforated in one or more points, inclosing pigmentary granulations, free pigment granules resulting from the destruction of the parasitic elements.

Richard* confirms fully the observations of Laveran, but instead of holding, like this observer, that the small pigmented forms are found adhering to the red corpuscles, he maintains that they, existing within the latter, where they are developed, grow and issue from them in a perfect state. He describes these pigmented parasites in various stages of development in the red globules, when arrived at a state of maturity perforating

* *Sur le parasite de la Malaria.* Comptes rendus (1882).

the membrane (?) of the red globule, and becoming free in the blood, presenting themselves thus sometimes furnished with movable filaments, like the bodies described by Laveran.

Both these last observers note that, in the organs of those dead from grave malarious infection, these supposed parasites are recognized by the *pigment*, which is arranged in circular series at their periphery.

It is right to observe at once that both these authors are entitled to the credit of having called attention to the pigmented elements, already to some extent noticed by others (Frerichs, Kelsch, &c.), which are not white cells of the blood; and to the second, viz., Richard, of having shown that the pigmented bodies are found in the red globules.

From this *résumé* of the history of melanæmia, it is clearly seen how the opinions of authors vary, not only as to the place of formation of pigment, but the precise way in which it is formed.

There is another category of authors, of whom some suspect the parasitic nature of the pigment granules; others hold these as partly constituting the parasitic element. It is therefore of some importance to return to the accurate study of melanæmia, and employ the means which the microscope has lately placed at our disposal.

This research could not be made with all the completeness desired, because for some years the malarious infection has in Rome and the Roman Campagna become mild, and consequently the grave forms of malaria have not been frequent in the hospitals, where, however, the slighter forms of intermittent abound. This diminution in the severity of the malarious infection has been further confirmed in the anatomical rooms, where in the bodies of peasants dead from other diseases it does not happen so frequently as in the past to meet with melanosis of the spleen, liver, and bone marrow—the melanosis which testifies to antecedent melanæmia.

The researches which we now describe were made in the blood of fever patients, taken at the Santo Spirito Hospital from July to the middle of October, 1883.

The method of examining the blood adopted by us was as follows:—

The blood was taken from a puncture, or small incision with a

ancet, in the finger of a fever case at different stages of the attack. The skin was previously washed with alcohol, and allowed to dry by evaporation. The first drop of blood was removed, and the succeeding ones used. On a drop of blood which welled from the wound a covering glass was delicately applied, so that a very small quantity might be deposited upon it; this was afterwards rapidly compressed against another covering glass, and they were then made to slide one upon the other, so that upon each remained a very thin film of blood, which was dried by passing it two or three times through the flame of a spirit lamp. Afterwards were dropped upon the preparation one or two drops of a recently filtered solution in alcohol and water of *methylin blue*, and after a few minutes washed thoroughly in a vessel containing distilled water, dried again, and the preparation mounted with oil of cloves, of citron, and balsam; the method being that used by R. Koch and P. Erlich.* The microscopic examination was made with a 1-12 in. (homogeneous immersion) lens, Zeiss.

We now set forth the result of the observations.

The normal red globules are stained faintly blue, and if the solution has only acted for a short time, or perhaps according to the degree of desiccation, remain of their usual yellowish colour.

The white globules exhibit a pale blue staining of their protoplasm, and an intense staining of the nucleus. The "*piastrine*"* are stained blue, a little less pale than the protoplasm of the white cells. But in the blood of persons affected with malarious infection, are found in the midst of the normal red globules, other red globules, which present alterations in their protoplasm, most evident in those which are not stained blue. These alterations consist in the presence within the globule of corpuscles in varying number, of different size and form, which are conspicuous from their more or less intense blue colour, always sufficiently to be distinguishable readily from the protoplasm, coloured or not, of the red globules. These corpuscles, advancing from the smaller to the larger, are seen—

(*a*) As granules, generally round, which are stained uniformly and more or less deeply blue, and often resemble micrococci. Of these one or more are found in a blood globule; in such

* *Elementary Granulations* (Bizzozero). *Blutplättchen* (German), a third cell element in the blood, according to Bizzozero.—E. D.

cases about two-thirds of the red globules present one, two, rarely three or four, of these corpuscles. Their size varies; some appear as very small granules, and others as a large micrococcus.

(*b*) As corpuscles larger than the preceding, with a vacuole in the centre so as to represent rings, more or less large and delicate. Of these rings some are found large enough to occupy one-third, or even one-half, of a red globule, sometimes spherical, sometimes oval, irregular or dentated, sometimes having a fine prolongation which terminates free in the protoplasm of the red globule or unites with an adjacent ring. One, two, three, or even more such are found in one blood globule.

(*c*) As corpuscles larger than the first, uniformly stained or with vacuoles, in form spherical, oval, spindle-shaped, semi-lunar, triangular, sometimes forming irregular masses which present in their interior granules or masses of black or rusty black pigment. The larger masses invade, in some red globules, all the protoplasm coloured by hæmoglobin, of which there remains either a delicate outline or a more or less subtile rim at some point of the periphery, scarcely visible with the most careful examination. Besides the red globules thus altered are found coloured bodies like the masses described, rich in clusters and granules, spherical, oval, irregularly semi-lunar. These evidently represent the last stage in the alteration of the red globule, which is thus converted into a body devoid of hæmoglobin, stainable with methylin blue, and containing pigment. Besides these red globules, of which the phases of change may be followed up to their conversion into a pigmented mass, there are found in preparations fragments of the latter in various forms, always pigmented, and white globules, in whose protoplasm pigment is found in granules and larger clusters than those found inclosed in the red globules.

If the blood issuing from the wound is mixed with a drop of pure distilled water, and then dried as in the first method, there are seen, together with normal red globules, which have lost their hæmoglobin and are only marked by a faint outline, others which have also lost their hæmoglobin and are distinguished by this faint outline, but which contain in their interior the corpuscles mentioned, stained deeply blue and clearly defined.

We have stained the blood with other aniline colours, acid and

basic, and here is the result. With *cosin* the corpuscles are stained pale rose, while the rest of the red globule is intensely yellowish red; the pigment remains unchanged. With *vesuvin* they stain red-brown. With *tropæolin* they are not stained at all, but are recognized, in the red globule stained a beautiful clear yellow, as so many spots containing pigment or not.

Examining the fresh blood without any treatment, we see only the larger corpuscles appear as colourless and pigmented specks, as already described by Richard. The red globules in which are found the corpuscles (small, round, analogous to micrococci) appear normal, or if there are specks, these are indistinguishable from the lacunæ which are formed accidentally in normal red globules.

The pigmented spots present various shapes, increase gradually, and coalesce, so that the entire red globule is converted into a decolorized body of hyaline aspect, containing granules and masses of pigment. When it contains one or two pigmented corpuscles and still retains a distinct portion of its coloured protoplasm, it preserves its normal elasticity. When it is converted into a hyaline mass it sometimes preserves its elasticity; sometimes this is diminished, and the globule adheres to the slide or covering glass, does not move, or only moves slightly when pressed.

The pigmented granules, which are found in the spotted globules as well as in those completely decolorized, assume various figures when the globules move, viz., circles round the periphery of the specks, circular groups or irregular forms. When the globules are at rest, the pigment granules are motionless or move actively, especially those found in the serum on the blood clot.

In preparations of fresh blood other forms of red globules are encountered, viz., such as present only one half decolorized and of a semi-lunar form, having in the centre or at a point of the periphery clusters of pigment; or such as have lost wholly or partially their hæmoglobin and exhibit in the centre a single spot of black pigment, rounded, irregular, having somewhat the shape of an embryo.

In addition are found round, hyaline, or irregularly pigmented bodies and richly pigmented white cells. On adding distilled water, the altered red globules first become shapeless, and

then assume a perfectly spherical figure, like the other red globules.

With the reaction of acetic acid and alcohol these altered red globules are recognized by the pigment which they contain; with liquor potassæ the pigment dissolves slowly after ten to fifteen minutes. Ferrocyanide of potassium and hydrochloric acid do not produce their characteristic reaction on the pigment.

Such is the result of the examination of the blood in persons affected with malarious infection, especially during the fever paroxysm.

Is this alteration of red globules always found in malarious infection? To this we must reply, that if in many cases the altered blood globules are very numerous, in others they are very scarce, so that it is necessary to make many preparations in order to find them; in other instances they have not been found at all. The latter has been the case in simple intermittent, and in some forms held clinically to be graver manifestations of malaria.

In *comatose pernicious fevers* terminating fatally we have always found it, as also in the *sub-continued* forms.

When we have found such appearances in the red globules the individuals always presented a peculiar aspect in the course of the disease, viz., the skin became rapidly *earthy yellow*, the weakness was extreme, and the strength re-established slowly and with difficulty.

The relation of the degree of alteration of the blood to the clinical course of the infection will be an interesting subject of study.

We come now to speak more particularly of the alterations found in the blood and organs of persons dead from pernicious fevers.

We shall only speak of the cases which have occurred this year, *i.e.*, after we had studied the alterations in the red globules during life, and give them as a whole, prefacing a note of the history and examination of the blood before death.

1st Case. *Algide Pernicious Fever.*—R. D., ætat 25, entered the hospital August 11th, suffering from intermittent fever (quotidian), and had several paroxysms in the hospital. 14th, at evening visit in a state of profound collapse; skin very cold, cyanotic, and pulse almost imperceptible. Hypodermic injec-

tions of quinine, which had been administered internally on previous days. Died next day at 2.45. A.M.

Examination of the fresh blood during life showed numerous white globules much pigmented, red globules not visibly altered.

After staining, many red globules showed in their interior, one, two, rarely three corpuscles, of which a few were small, and more or less deeply stained, others somewhat larger, with a vacuole in the centre, appeared as blue rings.

Autopsy (7 A.M., same day).—Persistent cadaveric rigidity. Skin pale; brain and meninges exsanguined; grey substance pale; heart normal; myocardium greyish red colour; lungs slightly œdematous; spleen doubled in size, with numerous recent peripheral hæmorrhagic infarcts; parenchyma soft and dark red; liver normal in size, with icteric staining of central parts of lobules; kidneys, capsule readily detached, surface greyish, *glomeruli* not prominent, pale, substance of convoluted tubes yellowish grey; numerous hæmorrhages of the pelvis and calices; stomach, mucous membrane swollen and hyperæmic, with numerous scattered hæmorrhages; intestine normal; bladder dilated.

The blood of various vascular areas and from the splenic pulp and bone marrow collected, and stained preparations made. These showed under the microscope numerous red globules, containing the corpuscles observed during life; some larger ones containing pigment; numerous nucleated red globules.

The microscopic examination of the various organs hardened with alcohol, and stained with Bismarck brown or methylin blue, showed in blood vessels, chiefly in the capillaries, the corpuscles described, deeply stained; if the red globules are recognizable by their contour, these corpuscles are seen distinctly inside of them; but if the globules were disintegrated and reduced to the well-known yellowish-grey *detritus*, the corpuscles were equally distinctly seen scattered through this *detritus*, and therefore not destroyed like the red globules. In the capillaries of various organs, and chiefly the brain, these corpuscles were at so regular a distance from each other as to make it evident that they were still within the red globules, even when the contour of these was indistinct. In all the vessels were found in addition pigmented white globules and pigmented bodies stained blue, representing the last change of red globules.

2ND CASE. *Comatose Pernicious Fever.*—P. E., ætat 40, entered the hospital September 29th, in the afternoon, in an *accès* of comatose perniciosa. There was no previous history, only that he came from the Campagna. He died next morning.

Microscopic examination showed scattered pigmented white cells; red globules with pigmented corpuscles; numerous hyaline bodies of different shapes, spherical, oval, fusiform, semi-lunar, containing granules and pigment masses. Some of these hyaline bodies had at their periphery a zone of protoplasm coloured with hæmoglobin; a certain semi-lunar form was the result of the conversion of one-half a red globule into a pigmented hyaline mass, whilst the other remained decolorized, and only its delicate outline was visible. The examination made shortly before death showed a great number of pigmented white globules.

The stained preparations presented numerous red globules containing round corpuscles either in centre, at periphery, uniformly stained, with a vacuole in the centre, or blue rings of various size and form.

Autopsy (7 hours after death).—Œdema of the membranes; punctiform hæmorrhages of white cerebral substance; grey substance dark red; bilateral pulmonary œdema; hydrothorax; hydropericardium; heart normal; myocardium brownish red colour; spleen triply enlarged, of triangular form, very soft, of a chocolate colour; liver dark grey colour; lobules indistinct; kidneys dark grey colour, exsanguined.

The result of the microscopic examination was the same as during life. As to the examination of organs hardened and stained, it deserves to be recorded that the capillaries of the brain were distended with red globules, containing the corpuscles mentioned, deeply stained, and without trace of pigment. The pigmented white globules were rarely met with in these capillaries.

3RD CASE. *Comatose Pernicious Fever.*—M. L., a carter outside the Porta Maggiore, entered the hospital at 10 A.M., Oct. 6, already profoundly comatose and with high fever; said to have suffered some days from fever, and found that morning in this condition in a stable. After injection and internal administration of quinine he rallied somewhat, reacted to cutaneous stimuli, but did not recover his senses. After a little he sank into profound coma. Had very grave dyspnœa; pulse 140; temperature

39°; dirty yellowish-grey colour of the skin; spleen swollen and tender; tracheal râles. Died at 6 P.M.*

Examination of fresh blood: white pigmented globules in great number, a few red globules with clear spots containing granules of black pigment. Examination after drying and staining: in about half the red globules were found either corpuscles perfectly spherical, stained intensely blue, or larger ones with a vacuole in the centre, presenting the aspect of rings of varying size and shape, some furnished with a prolongation terminating free or united to the next ring.

Autopsy (12 hours after death).—Body that of a robust man, well nourished; colour of skin livid yellow; ocular conjunctiva slightly icteric. On the internal surface of the dura mater corresponding to the right frontal lobe were found two clots inclosed by delicate membranes furnished with numerous small blood vessels. Two other hæmorrhages having the same characters corresponded to the left central convolutions.

Hæmorrhagic infiltration of the *meninges* of the occipital lobes; punctiform hæmorrhages of centrum ovale of occipital lobes; grey substance leaden red colour; grey substance of the ganglia presented the same in a minor degree, as did the grey matter of the pons, bulb, and spinal marrow; heart normal form and volume; myocardium brownish, lacerable; lungs congested and œdematous posteriorly; spleen triply enlarged, capsule tense, parenchyma dark chocolate, very soft; lymphatic glands of the hilum dark red, swollen, very soft; kidneys pale, flaccid; stomach and duodenum with numerous mucous hæmorrhages; liver normal size, rich in dark blood; surface on section lavender red; gall bladder turgid with bile; intestines normal, except last part of the ileum, which presents spots of hyperæmic hæmorrhage, and soft swelling of the agminated glands; bladder, small quantity of turbid urine, containing, as found on chemical examination, albumen and bile pigment, red and white blood cells, granular casts of varying size, some convoluted.

Microscopic examination, same results as during life, but the corpuscles contained in the red globules more numerous. Splenic pulp and bone marrow rich in more or less altered red globules,

* Professor Marchiafava and Dr. Ferraresi have not unfrequently found such cerebral hæmorrhages, which explains the fact that hemiplegia is often left behind after attacks of comatose perniciosa.

and the latter contains a remarkably large number of nucleated red globules.

Spleen and marrow of these corpses served also for ascertaining if the black pigment which they contained gave the reaction with iron which had not resulted from the same pigment inclosed within the red globules. We used as the re-agent a solution of ferrocyanide of potassium (1 in 12) slightly acidulated with pure hydrochloric acid. Small fragments of melanotic spleen, dissected out with glass needles, and placed under the microscope with a drop of the re-agent, showed the reaction in some red globules, in very many white ones, in many globuliferous cells, of which some have only diffused staining of the protoplasm alone; others show the same reaction in the inclosed globules.* But nevertheless, after twenty-four hours of the reaction, the bulk of the granules and pigment masses lost their proper rusty black colour. The treatment was repeated with the same negative result in the liver and brain containing pigment. In these, however, was found a slight blue staining of the corpuscles inclosed in the red globules, whilst their contained pigment remained unaltered. The reaction was not assisted by a gradual increase of temperature up to 80° C. Perls † had already observed that all the black pigment in the liver and spleen did not give the iron reaction, as it was not given by the bile pigment, hæmatoidin, the choroid pigment, and its homologue in retinitis pigmentosa.

From these three characteristic cases of malaric infection terminating fatally we have a confirmation of the existence of the alteration of red globules, especially from the fact that the capillaries of various organs, above all the brain, were seen to be gorged with red globules containing corpuscles, in two instances almost all primary, round, small, and not having any pigment granules.

It is noteworthy also that these corpuscles were not free, but, on the contrary, always enclosed within the red globules, and only appearing free when their contour was lost after the action of alcohol.

From what we have stated, it is clearly shown that in malarious

* Compare *Studi pathologici e chimici sulla funzione ematopoetica.* Tizzoni e Fileti. Trans. R. Acad. Liucei, 1880—81, vol. x.

† Virchow's Archives, T. 39.

infection there occurs a change in the red globules, which can be followed in all its phases; which begins with the presence of granules or corpuscles, generally spherical, stainable with any aniline dye, progressing with enlargement of these bodies, with their fusion, and the formation of blackish pigment in the form of granules and clusters, and ending in the reduction of the red globule into a pigmented body of a hyaline aspect, which is afterwards destroyed, and the pigment, set free, is inclosed in the white cells, and by them deposited in certain organs (spleen, bone marrow, liver).

Now what is the nature of this change in the red globules? That the change is to be regarded as of a retrogressive nature does not admit of doubt. We may, with Tommasi-Crudeli, define it to be *a necrobiosis of the red globule*, in which takes place the transformation of its *hæmoglobin* into *melanin*, whereby it only remains as a circulating *cadaver*, incapable of fulfilling its very vital function. This admitted, it may now be decided in reference to the site of formation and origin of pigment in melanæmia, 1st, *that the pigment is formed within the blood vessels and in the circulating blood;* 2nd, *that it is derived from the colouring matter of the red globule, and is formed absolutely in the protoplasm of the same.* The first conclusion is evidently admitted, that the pigment is formed in the circulating red globules, and agrees with what had already been established by Kelsch and Arnstein. It is intelligible from this reason alone why the hypothesis that the pigment is formed from extravasations of blood in the spleen and other organs cannot be received. The second conclusion is not less obvious, because whilst the red globule is decolorized, the black pigment is deposited, and this decolorization and pigmentation may be followed in all their phases.

Kelsch, as already mentioned, surmised that many of the pigmented forms represented decolorized and pigmented red globules, but abandoned this idea because he did not succeed in seeing all the steps of the pigmentation, and thought that the pigment was formed in the blood plasma from dissolved hæmoglobin.

As to the cause of such necrobiosis of red globules, we cannot at present say anything definite, any more than we can of other degenerations and necroses of elementary organisms in other

infectious diseases. To say that malaria attacks and determines the destruction of red globules, is only to repeat what clinical observation has already established. Bacelli * in fact long ago said that malaria struck not only the ganglionic system, whence the congestive processes from vasomotor paralysis, but also undermines the existence of the red globules, which lose their functional activity and perish. Besides the changes in red globules described by us, some other peculiarities seen are deserving of attention. The commencement of the change is indicated by the appearance in the red globules of granules and corpuscles generally spherical, which are deeply stainable by any aniline dye. Might these corpuscles represent micro-organisms which invade the red globules and penetrate their protoplasm?

No hypothesis could well be more seductive, but these characters do not suffice to make it acceptable. The granulations of the *mastzellen* of Ehlich present characteristics analogous to micrococci, and are yet only granulations of the protoplasm.

We have tried to settle the question by trying to cultivate the blood of the malarious, to see if these corpuscles would be multiplied.

The first attempts made had no result. Thus the cultivation of blood in Koch's gelatine produced no result, although the surrounding conditions were varied in many ways. The temperature of the hot box was, in the first experiments, maintained at fever heat; but in later experiments was, by the advice of Professor Filehne, who happened to be in Rome, kept at the normal of the human body, and gradually lowered to below 30° C. Blood was selected for cultivation in which the red globules showed the commencement of change, viz., very small corpuscles. The other conditions necessary for success were in the greater number of instances well attained, but round the little drops of blood and elsewhere not the slightest development of micro-organisms took place. Under like circumstances no result followed repeated cultivations, made in ossihæmoglobulin, prepared according to the directions of Hoppe Seyler and furnished by Professor Rossoni. After the failure of these attempts, others were made on the assumption that these supposed parasites might require, in order that their development might be

* La Perniciosa. Clinical Lectures, 1869.

seen, to be furnished with a soil of cultivation analogous to that of the red globule.

The necessity for using sterilizing temperatures, without at the same time altering the hæmoglobin, led to these ending in failure.

Finally, by a long process, resulted the preparation of a solid soil of cultivation answering to the conditions mentioned. In this was placed a drop of blood, taken with every precaution from the finger of a young man in the commencement of the cold stage of an ordinary intermittent. The hot box was kept at 36°—36°·5. During the first three days no change was seen round the tiny drop, but on the fourth day began to be seen a greyish-coloured halo, which was, on examination, found to be composed of round corpuscles of varied size, the larger colourless in the centre and resembling rings. Notwithstanding the apparent identity of the forms seen in the cultivation with those found in the blood of certain cases of perniciosa, we do not think any conclusions can be drawn from them. We only refer to the fact, which requires further research for its elucidation.

After the researches of Klebs and Tommasi-Crudeli,* bacillary forms were described by one of us (Marchiafava) and Cuboni as existing in malarious blood, swollen at the extremity and sometimes also in the middle, of varied length, and endowed with very active wriggling movements and locomotion. Afterwards the same forms were described by Marchaud† and by Ziehl.‡

Marchaud found in the blood of a fever patient bacillary forms with slightly swollen extremities, having about half the length of a red globule, and endowed with active movements. Ziehl found in the blood of three fever patients small bacillary forms, swollen at the ends, moving gently, but also found them in the blood of a diabetic who had no fever, but was thought to have latent malarious infection. In all four cases these disappeared under quinine. Subsequent observations showed that the parasitic nature of these was only apparent. Many such are figured in books on histology (Frey, Ranvier, &c.), and are well-known

* *Loc. cit.*

† *Kurtze Bemerkung zur ætiologie der Malarie.* Virch. Arch., 1880.

‡ *Einige Beobachtungen über den Bacillus Malariæ. Cl. Me. Woch.*, 48.

to depend on the mode of preparation and chiefly on the action of heat.

It is reasonable to hold, as already noted by Tommasi-Crudeli, that many of the forms described in the blood of malarious persons are due to filaments of this kind issuing from the blood corpuscles, and liable to impose on the observer as bacillary forms.

The small forms described by Marchaud and Ziehl remain to be more fully studied, especially as they have never been able to stain and preserve preparations of such.

The filaments described by Laveran and Richard, representing, according to the former, the parasite in its perfect state, originate in the way just mentioned. We have found in fresh blood that when the degeneration of the red globule is almost complete, and the normal protoplasm only remains as a delicate zone, very movable filaments separate from it, which after a time become free. These are never stained with aniline dyes, and however careful our examination we never discovered coloured prolongations in the degenerate and pigmented globules. The semi-lunar forms of Laveran originate when only half a red globule is converted into a pigmented semi-lunar body, whilst the other half is decolorized and only retains its subtile outline. Leaving these pseudo-organisms we come to the last part of our observations this year.

We have seen how in certain cases, from the cause described, an enormous quantity of red globules perish. We must add that in the same patients in whom this destruction of red cells occurs are found *red nucleated globules, larger red globules (macrocytes), and red globules which are coloured differently from ordinary ones.*

We cite the following case in point:—

C. L., ætat 19, of Rome, carter, entered the hospital September 10th. He had never previously suffered from malarious fevers. From September 1st to 8th he had attacks of quotidian intermittent. From the 8th he said the fever had never left him. A few hours after admission the fever was very high (41°), skin earthy yellow, spleen swollen and tender, sensorium blunted. Examination of the blood shows many red globules altered as described, pigment white ones. Quinine injected. Next morning temperature 38°·5, evening 40°. New injection of quinine.

Blood examined with same result. Subcontinued form lasted until the 15th, when the evening temperature was 37°·7. Blood examined with same result until the 13th, when there began to appear *nucleated red globules, very voluminous red globules*, with a diameter nearly twice that of a normal red globule. The number of the white cells and of the piastrine was clearly increased. Improvement continued until he left the hospital cured on the 28th, and whilst the altered and pigmented red globules and pigmented white cells went on diminishing, the nucleated red globules increased, and the macrocytes in their turn also became few in number. The convalescence was rapid. The appetite become voracious. The vital powers, which were reduced, returned quickly.

The red nucleated globules and macrocytes in many cases of acute malarious infection are recognized in the fresh and in the dried and stained preparations. In those stained with methylin blue the nucleated red globules exhibit their protoplasm greenish blue in colour, the outline and corpuscles of the nucleus dark blue. The form varied; protoplasm sometimes abundant, sometimes scarce. Usually they have one but sometimes two nuclei, one in process of division or germination.

This nucleus often seems issuing from the protoplasm. The macrocytes, which in the fresh state are very pale, are coloured by methylin blue less intensely greenish blue than the nucleated red cells.

The "piastrine" are coloured slightly blue, and appear alone or in groups, almost always with sharp outlines.

What is the meaning of the presence of all these elements, and especially of the nucleated red globules?

It is known that the nucleated red globules are not normally found in the circulation in extranterine life except in the first days of the same, but are found sufficiently frequently in the grave essential and symptomatic anæmias, in more or less considerable number, whilst they are very abundant in the bone marrow, and even spleen.* Erlich† drying and staining blood preparations found them easily. Hitherto, however, they have

* In a case of grave anæmia in a young person Marchiafava found numerous nucleated red globules (10—12 in each preparation), and after death a large number in the spleen and bone marrow.

† *Berlin Klin. Woch.*, 1881.

not been described in acute malarious infection. Their presence, as well in the later stages of the disease as during convalescence, has without doubt a connexion with the destruction of red globules, and is a sure indication of the greater energy of function of the hæmopoietic organs rendered necessary by the destruction of red globules in such large numbers.* As to the macrocytes and red globules coloured differently to the ordinary ones, they are evidently young red globules which have recently lost their nucleus, and this coincides with what has been observed by Bizzozero and Salvioli† on the blood of animals rendered anæmic by frequent bleedings.

We wished by experiments, in which destructions of red blood globules had been artificially produced, to institute a comparison with those cases in which this was due to malarious disease. We selected pyrogallic acid as the agent for this destruction, as shown to be such by the experiments of Neisser, Afanassiew, &c.

1st Experiment.—White dog of medium size.

3. X. 83. 11 a.m., subcutaneously injected with gr. 0·5 of pyrogallic acid dissolved in 10 cc. of distilled water. 7 p.m., animal not at all ill.

4. At 11 a.m., injected gr. 1 pyrogallic acid in 10 cc. distilled water. 1 p.m., blood examined. Numerous red globules in process of destruction, or reduced to simple decolorized bodies. Masses of hæmoglobin of varied size, staining with *eosin*. Animal collapsed. Urine olive green. No jaundice. Died at 5.30 p.m.

Autopsy.—All the viscera of a leaden grey colour; spleen swollen and black; liver dark blue; gall bladder surcharged with fluid bile; kidney same colour; bladder empty; bone marrow chocolate; black coagula in heart and blood vessels. Blood examined, shows red globules undergoing destruction; numerous red globules perfectly decolorized; granules and masses of pigment, and some nucleated red globules. Splenic pulp and bone medulla, especially the latter, show numerous nucleated red globules.

* Professor Rossoni has often observed with the spectrum of Vierordt the gradual diminution of hæmoglobin in the blood in relation to the quantity of altered red globules, and its gradual daily increase in convalescence along with the appearance of numerous nucleated red globules in the blood.

† *Ricerche sperimentali sulla Ematopoesi splenica.* Archiv. Bizzozero, vol. iv. n. 2.

2ND EXPERIMENT.—Black dog, middle size.

5. X. 83. 10 A.M., subcutaneous injection of gr. 0·5 pyrogallic acid in 10 cc. of distilled water. 1 P.M., examination of blood negative.

6. 7 P.M., examination of blood. A few red globules undergoing destruction.

7. 10 A.M., injection as above. 11 A.M., blood examined. Many red globules in process of destruction; a few nucleated red cells. Animal much depressed. Urine dark olive. Microscopic examination of urine shows casts of hæmoglobin. No jaundice.

8. 11 A.M., blood examined. Small number of red globules in process of destruction; nucleated red globules. Animal gradually recovered up to 15th, when no altered red globules nor nucleated red globules were found.

3RD EXPERIMENT.—Small black dog.

11. X. 83. 12 noon, subcutaneous injection of gr. 1 pyrogallic acid in 10 cc. of distilled water.

12. 8 A.M., dog collapsed—somnolent. Does not eat. Urine olive green; vomits yellowish-green liquid streaked with blood; sclerotic, not jaundiced. Blood examined; numerous red globules in course of destruction; cells decolorized; masses of hæmoglobin. 5 P.M., same state.

13. Blood examined. Same as above. Animal continues sunken—somnolent. Does not eat.

14. Examination of blood fresh and after staining. Very few nucleated red globules; red globules very much larger than normal. Dog in same state.

15. Examination as above. Animal collapsed.

16. 9 A.M., found dead.

Autopsy.—All the organs leaden grey colour. In every dried and stained preparation of blood of right heart numerous red globules in course of destruction; normal red globules; red globules larger, and even double normal diameter; red globules normal in size, stained greenish-blue; a few nucleated red globules (4—5 per preparation); numerous white globules, less or more nucleated with protoplasm, granular and hyaline; endothelial cells. In the splenic pulp numerous white cells, containing coloured fragments of red blood globules and nucleated red globules. The bone marrow, microscopically of dark red

colour, contained white cells inclosing fragments of red globules; proper cells of marrow; cells with budding necleus, and nucleated red globules in greater quantity than in the spleen and blood of the heart. The nucleated red globules both in the circulating blood and in the spleen and marrow showed more distinctly by staining with methylin blue the preparation dried by heat.*

From these experiments it results, therefore, that by producing destruction of red globules, after a very short time are found in the blood nucleated red globules; red globules of more than normal size; globules staining differently to ordinary ones—elements all met with more abundantly in the spleen and bone marrow after death. That the presence of such nucleated red globules is derived from augmented hæmopoietic activity, and expresses a true regenerative compensation, is beyond doubt. The same explanation is given by all who have of late studied the physio-pathology of the blood.

This view is strongly confirmed by our observations, because in some cases of malaria infection one is almost able to see before his eyes the two processes of destruction and regeneration of red globules.

Thus by examining the blood of a subcontinued fever patient, from the day of his entrance into hospital to that on which he left it cured, we see that three or four days after the destruction was arrested nucleated red globules began to appear, increased rapidly in number, and once more diminished as convalescence became established.

It is reasonable therefore to hold that the passage into the circulation of nucleated red globules happens, because the formation of red globules in the hæmopoietic organs is so active and hurried that the red globules, in course of formation, are forced into the circulation before they have lost their nucleus.

Although these researches of ours have not yet fully attained their aim, we believe we have helped to make better known the change produced in the blood in malaria, and to clear up the origin of melanæmia. It still remains to determine the nature of the specific agent, to which these changes are due, and to this end further researches will be directed. Finally, it will be easy to judge if any advantage is likely to accrue from them to clinical medicine.

* Bizzozero, Neumann, Salvioli, Foà, Pellucani, Orth, Lillen, &c.

It has been already said that they are not always found in slight, and are also absent in some grave forms, clinically considered to be malarious, therefore their absence does not exclude malarious infection, while their presence does not render it indisputable; and, furthermore, it has not occurred to us to find them in other infections (typhoid fever, cerebro-spinal meningitis, measles, pneumonia), in which we have had an opportunity of examining the blood.